involved with tumor target recognition (Farag et al *Blood*, 2002). In particular, AML and Neuroblastoma are NK sensitive pediatric tumors. CB is limited by the absence of available donor effector cells (NK, CTL, LAK and NKT cells) for infusion after UCBT (Cairo et al *Transfusion*, 2005). We demonstrated the ability to EvE CB in short-term culture with IL-2, IL-7, IL-12 and anti-CD3 with increased $CD3^-/16^+/56^{+dim\ and\ bright}$ subsets expressing KIR3DL1, KIR2DL2, KIR2DL1/S1 and CD94/NKG2a with increased NK and LAK cytotoxicity (Ayello/Cairo et al *BBMT*, 2006). In this study, we compared short-term culture (48 hrs) with prolonged cultures (4-10 days) on expansion of NK cells expressing NCR, KIR, NKG2, lytic ability and mechanisms of tumor lysis. Rethawed CB cells were cultured 2-10 days with anti-CD3 (50 ng/ml), IL-2 (5 ng/ml), IL-7 (10 ng/ml) and IL-12 (10 ng/ml) [ABCY]. NKR expression (KIR2DS4, NKG2D, CD94, NKp46), intracellular granzyme B and LAMP-1 receptor (CD107a) expression were determined by flow cytometry. Cytotxcity of EvE effector CB cells was measured by europium release assay at 20:1 E:T ratio with tumor targets K562 (NK), Daudi (LAK), Kasumi-1 (AML) and SYSY5Y (neuroblastoma). KIR2DS4 was significantly increased at day 10 vs 2 in ABCY in both $CD3^-/16^+/56^{+bright\ and\ dim}$ subsets (16.9±0.4 vs 2.1±0.2% and 22.3±0.3 vs 0.9±0.2%, $p<0.001$, respectively). C-lectin receptor CD94/NKG2D expression was increased at day 7 vs 2 (41.4±0.43 vs 23.7±2.0%, $p<0.001$). NCR expression in $CD3^-/16^+/56^{+dim}$ NKp46 subset was increased at day 7 vs 2 (10.1±0.06 vs 2.62±0.8, $p<0.001$). Granzyme B expression was increased from day 2 to 10 (25.8±1.7 vs 45.1±1.7%, $p<0.001$). CD107a expression was significantly increased at day 7 vs 2 (12.9 ±1.4 vs 69.3±2.2%, $p<0.001$). Additionally, increased cytotoxicity was demonstrated at day 7 vs 2 with tumor targets K562 (71.5±0.81 vs 53.8±3.9%, $p<0.001$), Daudi (63.9±0.73 vs 38±1.1%, $p<0.001$), Kasumi-1 (56.6±0.4 vs 31.8±1.8, $p<0.001$) and SYSY5Y (59.5±5.35 vs 32.6±4.9%, $p<0.01$). In summary, CB MNC may be thawed at time of transplantation, recryopreserved, rethawed at a later date, expanded and activated up to 10 days to yield viable NK subsets which appear to be cytolytic against AML and Neuroblastoma and could be potentially used as ACI post UCBT.

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EARLY HEMATOPOIETIC CELLS, INCLUDING MEGAKARYOCYTE PROGENITORS, ARE RECOVERED IN ALDH BRIGHT CELL POPULATIONS ISOLATED BY CELL SORTING FROM PREVIOUSLY FROZEN UMBILICAL CORD BLOOD

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ALDH bright [$ALDH^{br}$] cell populations sorted from fresh umbilical cord blood [UCB] on the basis of their high aldehyde dehydrogenase [ALDH] activity are known to include hematopoietic progenitor cells [HPC]. However, neither the hematopoietic potential of ALDHbr cells recovered from previously frozen UCB, nor the ability of any $ALDH^{br}$ cells to generate platelets has been reported. We have measured hematopoietic [CFC-H] and megakaryocytic [CFC-M] colony forming cells in $ALDH^{br}$ and $ALDH^{dim}$ [depleted of $ALDH^{br}$ cells] populations from thawed UCB. Cells were washed, immunomagnetically depleted of cells expressing glycophorin A and CD14, reacted with ALDEFLUOR® for flow cytometric detection of ALDH, and sorted to yield $ALDH^{br}$ and $ALDH^{dim}$ populations. $ALDH^{br}$ cells comprised 0.38 ± 0.15% [mean ± SD] of thawed UCB cells. $CD34^+$ and $CD133^+$ cells comprised 90.9 ± 3.1% and 57.7 ± 9.4 % of $ALDH^{br}$ cells, respectively. $CD41^+$ and $CD110^+$ cells were 23.4 ± 11.7% and 1.7 ± 3.4 % of the $ALDH^{br}$ population, respectively. CFC-H activity was enriched 1116-fold in the $ALDH^{br}$ population compared to the $ALDH^{dim}$ population [256 ± 104 colonies/1000 cells vs. 0.23 ± 0.33 colonies/1000 cells], and 65% of the CHC-H were recovered in the $ALDH^{br}$ population. The ratio of erythroid to myeloid colonies generated from $ALDH^{br}$ population was 3.7. Others have shown that most $ALDH^{dim}$ HPCs in fresh UCB are short term progenitors, whereas earlier long-term culture initiating cells and cells that establish long-term grafts in NOD-SCID mouse models are $ALDH^{br}$. Consistent with this, we found that GEMM colonies are about ten times more frequent in $ALDH^{br}$ CHC-H [6.6 ± 4.5 GEMM /1000 cells] than in $ALDH^{dim}$ [0.6 ± 1.2 GEMM/1000 cells] cells from thawed UCB. Furthermore, CFC-M activity was 2015-fold higher in the $ALDH^{br}$ population than the $ALDH^{dim}$ population [60 ± 35 colonies/1000 cells vs. 0.03 ± 0.02 colonies/1000 cells]. Cells giving rise to large megakaryocyte colonies, usually considered to be CFC-M with the most self-renewal potential, were particularly enriched in the $ALDH^{br}$ populations; all the large colonies we detected [38.0 ±17.4 large CFC-M/1000 cells] were derived from $ALDH^{br}$ populations. The rare CFC-M colonies from $ALDH^{dim}$ cells were small or non-megakaryocytic. These results suggest that $ALDH^{br}$ cells recovered from thawed, banked UCB could be used to reconstitute erythroid and myeloid, including megakaryocytic, blood elements after transplantation.

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PD-1 IS REQUIRED TO INDUCE PERIPHERAL CD8 T CELL TOLERANCE IN RECIPIENTS OF ALLOGENEIC BONE MARROW TRANSPLANTATION WITH ANTI-CD154

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We examined the mechanisms involved in peripheral CD8 T cell tolerance induced by mixed chimerism achieved with non-myeloablative conditioning with low-dose (3 Gy) total body irradiation (TBI) and 2mg of anti-CD154 antibody. CD8 T cell tolerance is CD4 dependent and is characterized by a specific anergic state toward donor antigens prior to specific deletion of donor-reactive cells. We tested the role of the PD-1 pathway in a model in which only CD4 peripheral tolerance is required (i.e. TBI 3 Gy and depleting anti-CD8 mAb Day -1, 2mg of anti-CD154 Day 0 followed by allogeneic BMT) and in a model in which both CD4 and CD8 peripheral tolerance is required (i.e. TBI 3 Gy Day -1 and 2mg of anti-CD154 followed by allogeneic BMT) for the development of mixed chimerism. PD1-/- (C57BL/6 background) or C57BL/6 wild-type mice received fully allogeneic bone marrow cells (B10.A). While WT control mice showed successful mixed chimerism induction with both regimens, the PD1-/- recipients failed to develop mixed chimerism unless they were CD8 depleted. These results indicate that PD1 is required for the tolerance of peripheral donor-reactive CD8 cells but not for that of CD4 T cells. We confirmed these results using blocking anti-PD1 and anti-PD-L1 mAb in WT B6 recipient mice. While control groups again showed successful engraftment, recipient mice treated with blocking anti-PD-1 and anti-PDL-1 mAb failed to develop mixed chimerism unless they were depleted of CD8 cells. Chimeric mice that were CD8 depleted and received PD-1 blockade accepted donor skin grafts while rejecting third party grafts. Thus, a functional PD1-PDL1 pathway is critical to achieve donor-specific CD8 T cell tolerance in our model.

GVH/GVL

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BLOOD EOSINOPHILIA AS A MARKER OF FAVORABLE OUTCOME AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Eosinophilia is observed in a variety of systemic disorders including acute and chronic graft-versus-host disease (GVHD) after allogeneic stem cell transplantation (allo-SCT). The clinical records of 237 adult patients who underwent allo-SCT were retrospectively reviewed. Peripheral complete blood cell counts (CBCs) had been performed at least two or three times a week until day 100 in all cases, and white blood cell differentiation was

evaluated by microscopic examination. Eosinophilia was defined as a relative eosinophil count greater than 4% on at least one day within the first 100 days after allo-SCT. Eosinophilia was observed in 135 patients (57%). The cumulative incidence of grades II to IV acute GVHD was found to be significantly higher in patients without eosinophilia than in those with eosinophilia (68% vs. 43%; $P < 0.001$). In 15 of 58 (26%) patients with eosinophilia and 41 of 70 (59%) patients without eosinophilia, acute GVHD was resistant to standard doses of prednisolone and required salvage therapy ($P = 0.022$). The cumulative incidence of chronic GVHD was significantly higher in patients without eosinophilia than in those with eosinophilia (73% vs. 56%; $P < 0.011$). The cumulative incidence of relapse in patients with hematologic malignancies was similar between patients with and without eosinophilia (33% vs. 27%; $P = 0.438$). On the other hand, the cumulative probability of non-relapse mortality was 10% in patients with eosinophilia, which was significantly lower than that in patients without eosinophilia (31%; $P < 0.001$), and the estimated overall survival at 3 years was 67% in patients with eosinophilia, which was significantly higher than that in patients without eosinophilia (51%; $P = 0.003$). Multivariate analysis identified age above 40 years, high-risk disease, grade II to IV acute GVHD, sex disparity between patient and donor, and the absence of eosinophilia as significant factors for reduced overall survival. These data lead us to conclude that eosinophilia after allo-SCT may serve as a favorable prognostic marker. However, further prospective studies including detailed cytokine profiling are essential for an understanding of the pathophysiological mechanisms involved in posttransplant eosinophilia.

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A CRITICAL CONTRIBUTION OF DONOR -173G/C POLYMORPHISM OF MACROPHAGE MIGRATION INHIBITORY FACTOR GENE TO THE DEVELOPMENT OF CHRONIC GRAFT VERSUS HOST DISEASE

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Chronic graft versus host disease (cGVHD) severely impairs the clinical outcome and life quality after allogeneic stem cell transplantation (allo-SCT). Its pathophysiology is not fully understood, and treatment often fails. Macrophage migration inhibitory factor (MIF) is produced by various cell types including T cells, macrophages, epithelial cells and shows a broad range of proinflammatory properties. A G to C transition at position -173 of the MIF gene has been associated with the development of various inflammatory diseases. Here, we assessed the contribution of the minor C allele to cGVHD development.

Donor-recipient pairs of 405 patients receiving allo-SCT (matched unrelated donor (URD): n=225; matched related donor (MRD): n=180) at four independent centers and surviving >100 days after SCT were genotyped. Stem cells were derived from bone marrow (n=163) or from peripheral blood (n=242) and T cell depletion (TCD) was performed in 30.7% of cases. Mean follow up was 888±46 days. The C allele (GC or CC genotype) was present in 29.2% of recipients and 26.7% of donors. No association was seen between acute GVHD and the G/C polymorphism of either the donor or the recipient. Overall, 46.8% of all patients developed cGVHD: 43.1% of patients with a GG genotype donor and 55.4% of patients when the donor carried a C allele (p=0.04). The incidence of cGVHD was not altered by the patient's MIF status (GG: 45.3% vs. GC/CC: 49.1%; p=0.6).

In patients receiving URD SCT, the presence of a donor C allele resulted in an increase in cGVHD development from 47.2 to 63.9% (p=0.03). This effect was not seen in MRD SCT (p=0.58). Since T cells are a major source of MIF, we then tested the effect of TCD on the role of donor MIF in cGVHD development after URD SCT. cGVHD incidence was significantly increased in patients receiving non-TCD allo-SCT from GC or CC unrelated donors compared to recipients of GG donor cells (71.1 vs. 52.9%; p=0.045). When TCD was performed, cGVHD occurence was not affected, independent whether the donor carried the C allele or not (52.2 vs. 40.0%; p=0.2). The presence of a donor C allele further led to a significant increase in treatment related mortality (TRM) from 6.4 to 28.6% when no TCD was performed (p=0.002), whereas TRM after TCD in both groups ranged between 31.0 and 32.7%.

Collectively, our data demonstrate a distinct role for donor T cell derived MIF in the development of cGVHD, which may allow MIF as a new immunotherapeutic target in future.

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TREATMENT OF EXPERIMENTAL ACUTE GRAFT-VERSUS-HOST DISEASE USING EXTRACORPOREAL PHOTOTHERAPY: A NOVEL MURINE MODEL

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Extracorporeal phototherapy (ECP) is an emerging therapy for clinical graft-versus-host disease (GVHD) that exposes a patient's peripheral white blood cells (WBCs) to photo-activatable 8-methoxypsoralen (8-MOP) and UVA light before re-infusing them. We have developed a novel murine model that closely mirrors ECP treatment of clinical GVHD to investigate its mechanism of action. C3H.SW mice were conditioned with 11Gy total body irradiation and injected with 5.0×10^6 bone marrow and 0.5×10^6 purified T cells from either syngeneic (C3H.SW) or allogeneic (B6-Ly5.2) donors. In order to model ECP as a treatment strategy rather than as prevention, animals were not treated until GVHD was clinically evident 7 days after BMT and then received 4 weekly infusions with 30.0×10^6 8-MOP+UVA treated splenocytes from similarly transplanted mice. Control mice were infused with untreated splenocytes, and did not show any differences from mice treated with L-15 (data not shown). Infusion of 8-MOP+UVA-treated splenocytes into allo-BMT recipients resulted in significantly better day +56 survival (74% vs 42%, p=0.0007), reduced GVHD clinical scores (2.9 vs 5.9, p<0.004) and GVHD histopathology in liver (7.3 vs 13.4), gut (11.4 vs 18.4) and skin (0.6 vs 1.2; all organs p<0.03) compared to mice infused with untreated splenocytes. ECP treated allo-BMT recipients also had significantly better immune reconstitution (p=0.01) on day +56, with both total thymocytes and distribution of thymocyte compartments indistinguishable from syngeneic controls. In vitro studies showed that >98% of cells were Annexin$^+$ within 24h of ECP treatment and experiments using Ly5 congenic donors demonstrated that ECP treated cells are undetectable in the thymus and spleen following injection. Because apoptotic cells are known to induce tolerance in several systems, we hypothesized that ECP increased the number of T regulatory cells (Treg). The number of CD4$^+$CD25$^+$Foxp3$^+$ Treg in ECP-treated allo-BMT recipients was significantly increased compared to mice infused with untreated splenocytes in the thymus (2.5 vs 1.0×10^4, p<0.02) and the spleen (1.8 vs 1.1×10^5, p=0.008). We conclude that 4 weekly infusions of ECP treated cells beginning after GVHD induction significantly decreases acute GVHD by all clinical, pathological and cellular parameters and is associated with increased numbers of Tregs.

	SYN +/- ECP	ALLO+ No ECP Spl	ALLO+ ECP Spl	p-value*
Number	15	19	34	
Day +56 Analysis:				
Survival	100%	42%	74%	0.0007
GVHD Clinical Score	1.1±0.4	5.9±0.3	2.9±0.4	0.004
Pathology				
Liver	2.1±0.9	13.4±5.5	7.3±2.5	0.0001
Intestine	7.4±2.8	18.4±5.7	11.4±3.1	0.0003
Skin	0	1.2±0.7	0.6±0.6	0.03
Thymic reconstitution				
Thymocytes (x10^6)	12.4±9.9	2.2±2.1	7.7±7.2	0.01
Thymic Treg (x10^4)	5.1±2.6	1.0±0.6	2.5±1.4	0.02
Spleen Treg (x10^5)	6.2±2.9	1.1±0.4	1.8±0.1	0.008

* 95% confidence level, Allo+ECP Spl vs Allo+No ECP Spl